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(54) BIOCHEMISTRY SENSOR AND BIOCHEMISTRY DETECTION DEVICE USING IT

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a sensitive sensor array and a measuring device.

SOLUTION: In a biochemistry sensor, a macromolecular fine particle with a particle size of 5 nm-100 μm being modified by a reagent that contains at least one of an antibody, an antigen, a single-stranded DNA, a receptor, a ligand, and an enzyme is turned into a solid phase in a divided region on a flat substrate 1. The substrate 1 is divided into a plurality of regions, and in each region, one layer of a polystyrene fine particle being modified by a different organism molecule is adsorbed. When a specimen in a sample is modified by fluorescein coloring matter, and the sample is added to the substrate 1, protein 8 that is the specimen with specific combination capability to an antibody 4 is adsorbed to a polystyrene fine particle 3, and fluorescein coloring matter 9 is combined with a region 2. Excitation light 14 is applied, thus monitoring a fluorescein signal being generated by exciting the fluorescein coloring matter 9 or a fluorescein coloring matter 11 by a camera 17 via an optical system 16.

CLAIMS

[Claim(s)]
[Claim 1]A biochemical sensor, wherein particles of polymers with a particle diameter of 5 nm to 100 micrometers embellished with an antibody, an antigen, a single stranded DNA, receptor, ligand, and a reagent containing at least one of the enzymes are solid-phase-ized by classified field on a flat substrate.
[Claim 2]The biochemical sensor according to claim 1 with which particles of said polymers were solid-phase-ized on a substrate via metal thin films given to a substrate face, such as gold, silver, copper, platinum, and aluminum.
[Claim 3]The biochemical sensor according to claim 1 or 2 with which free electron metal thin films, such as gold, silver, copper, platinum, and aluminum, were given to a substrate face of particles of said polymers, and the surface in a left side.
[Claim 4]A biochemical sensor, wherein with a particle diameter of 1 nm to 100 micrometers embellished with antibody, antigen, single stranded DNA, receptor, ligand, and reagent containing at least one of the enzymes golden particles are solid-phase-ized by classified field on a flat substrate.
[Claim 5]An optical system which irradiates with parallel-ized white light, a biochemical sensor which can irradiate with said parallel-ized white light at an angle of predetermined, It consists of a measurement means which measures a spectrum of an optical system which detects light reflected from this biochemical sensor, and detected light, In said biochemical sensor, particles of polymers with a particle diameter of 5 nm to 100 micrometers are solid-phase-ized by classified field on a flat substrate, A biochemistry sensing device, wherein a thin film of free electron metal, such as gold given to a substrate face of particles of said polymers and the surface in a left side, silver, copper, and platinum, is embellished with an antibody, an antigen, a single stranded DNA, receptor, ligand, and a reagent containing at least one of the enzymes.
[Claim 6]An optical system which irradiates with parallel-ized monochromatic light, a

biochemical sensor which can irradiate with said parallel-ized monochromatic light at an angle of predetermined, It consists of a measurement means which measures an optical system which detects light reflected from this biochemical sensor, and detected luminous intensity, In said biochemical sensor, particles of polymers with a particle diameter of 5 nm to 100 micrometers are solid-phase-ized by classified field on a flat substrate, A biochemistry sensing device, wherein a thin film of gold given to a substrate face of particles of said polymers and the surface in a left side, silver, copper, or free electron metal of platinum is embellished with an antibody, an antigen, a single stranded DNA, receptor, ligand, and a reagent containing at least one of the enzymes.

[Claim 7]Construction material of particles of polymers and thickness of particle diameter and free electron metal particles which are solid-phase-ized differ from each other for every field, The biochemical sensor according to any one of claims 1 to 3 with which said antibody embellished by said each field, an antigen, a single stranded DNA, receptor, and ligand differ from a reagent containing at least one of the enzymes.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the biochemistry sensor chip which has the field embellished with biochemical reagents, such as two or more antibodies, an antigen, a single stranded DNA, receptor, ligand, and an enzyme.

[0002]

[Description of the Prior Art]A DNA chip is mentioned as a Prior art. As shown in drawing 2, DNA of several 10 mer(s) which are different to a majority of two or more fields is combined by using the art of optical lithography and a photochemical reaction on the substrates 30, such as silicon and glass. For example, the 1st DNA32 is combined with the 1st field 31, and the 2nd DNA34 is combined with the 2nd field 33. After using as the short fragment of several 10 mer(s) the gene which is analyte and embellishing each with a fluorochrome in detection, the sample of analyte is added on the surface of a DNA chip. Those fragments will be combined if DNA currently compounded on the DNA chip and the DNA fragment which has a complementary relation exist in a sample. For example, DNA fragment 35 which has a complementary relation DNA34 is combined with DNA34. If a DNA chip is observed by the high sensitivity photodetection methods, such as the confocal microscope 36, after flushing other DNA fragments which are not combined, the fluorescence signal 38 from the fluorochrome 37 combined with DNA fragment 35 combined with the specific region will be detected. Since DNA combined for every field is decided beforehand, the fragment of DNA is

identified from the field where the signal was detected.

[0003]

[Problem(s) to be Solved by the Invention]In the above-mentioned conventional technology, while it is a method very advantageous to detecting a huge kind of DNA promptly, it cannot use for detection of other biomolecules, such as an antibody, an antigen, receptor, and ligand, easily.

[0004]The DNA fragment in a sample is embellished with a fluorochrome, and since comparatively complicated optical systems, such as a confocal microscope, are required, it is hard to become a cheap device detecting a signal.

[0005]

[Means for Solving the Problem]In order to solve an aforementioned problem, creation of a sensor chip uses a surface chemistry patterning method for polystyrene particles embellished with said biomolecule on a golden board.

[0006]About a detecting method, artificers use coupling considered to originate in an interaction of surface plasmon of a gold thin film discovered recently and golden particles.

[0007]

[Embodiment of the Invention]It proposes arranging on a substrate the polymers minute ball or golden particles which were already embellished with biomolecules, such as DNA, an antibody, an antigen, receptor, ligand, and an enzyme. As a basic principle, the following phenomenon discovered recently is used about polystyrene particles. If polystyrene microspheres with a particle diameter of 5 nm to 100 micrometers suspended in the carbodiimide liquid of 1 to 50mM are added to said gold thin film, the particles of one or less layer will be formed in the gold thin film surface. Therefore, in order to perform pattern NINGU of polystyrene microspheres, Make particles adsorb selectively on the gold pattern formed on the substrate, or. Or it becomes possible by forming the pattern of a thiol molecule by the art of lithography on a uniform gold thin film, making polystyrene particles adsorb on said pattern, or preventing adsorption by said pattern top to arrange the particles of polystyrene on a substrate. A method which used the optical exposure about patterning of a thiol molecule (from the 626th page to 628 pages [Langue MYUA 10 (1984)] (Langmuir 10(1984)pp626-628)), Or the method (from the 696th page to 698 pages [Science 264 (1994)] (Science 264(1994)pp696-698)) using the stamp of rubber is known. The scanning electron microscope photograph of drawing 3 shows an example of the patternized polystyrene particles. When the hole of a hexagon whose one side is 400 nm placed the metallic mesh formed regularly on a polystyrene substrate and vapor-deposited gold 20 nm in thickness, the gold pattern of the hexagon was formed. By adding a particle with a particle diameter of 110 nm suspended in the carbodiimide liquid of 1 to 50mM to a gold pattern, polystyrene particles stick only to up [of a gold pattern], and it looks white by drawing 3.

[0008]In adsorbing precious-metals particles, the following method is used. A gold thin film is formed by vacuum evaporation on a substrate. If the suspension of the alkanethiol molecule which has an amino group is added on the gold thin film surface, the monomolecular layer of an alkanethiol molecule will be formed in the gold thin film surface. Next, by adding the carbodiimide liquid of 1 to 50mM to the suspension of the alkanethiol molecule which has a carboxyl group, and adding on said gold thin film surface, The gold thin film embellished with the sulfhydryl group can be obtained (from the 1865th page to 1868 pages [Langue MYUA 13 (1997)] (Langmuir 13(1997)pp1865-1868)). Said sulfhydryl group adsorbs precious-metals particles easily. Therefore, in order to pattern a precious-metals minute ball, The gold pattern formed on the substrate by a sulfhydryl group by the above-mentioned method After ornamentation, Precious-metals particles are made to adsorb selectively, or the pattern of a thiol molecule is formed by the art of the above-mentioned lithography on a uniform gold thin film, and it becomes possible also by adsorbing a precious-metals minute ball on said pattern. The sample which adsorbed gold colloid on the gold thin film is shown in drawing 4. When the polystyrene particle with a particle diameter of 5 microns could be regularly stood in a line on the polystyrene substrate and gold was vapor-deposited from the top, the gold pattern of the triangle whose one side is 2 microns was formed. After embellishing this gold pattern with a sulfhydryl group by the above-mentioned method, when gold colloid liquid with a particle diameter of 100 nm is added, it turns out that gold colloid adsorbed selectively on the gold pattern.

[0009]In order to detect adsorption of biomolecule simple, the following phenomenon discovered recently is used about the optical property of the metal particles formed on the metal thin film. If 500 nm of gold is vapor-deposited from 5 nm in thickness on the polystyrene particles solid-phase-ized on the substrate, golden hat-like particles will be formed on polystyrene particles (Japanese Patent Application No. 9-148935). By having formed golden particles comes (Japanese Patent Application No. 9-148941) to show coloring with a remarkable substrate. Since this coupling does not have wavelength dependency to the transmitted light while producing by absorbing the light of some wavelength band regions, when white light is irradiated with a definite angle to a substrate and reflected in a substrate face, a development characteristic is not observed. The absorption maximum wavelength of a reflection spectrum can be used as a sensitive sensor from shifting depending on the refractive index in the field of 100 nm or less on the golden particle surface.

[0010]It is thought that this coupling originates in the interaction of the surface plasmon of a gold thin film and golden particles. If it irradiates with the usual real ball-like gold particles by white light, the surface plasmon which is collective vibration of the free electron in particles will be excited. Since have different dispersion relation from the surface plasmon in a gold thin film and it is not spread, it is called local surface plasmon. A resonance wavelength is dependent on the particle diameter and shape of particles

(from the 678th page to 700page [Surface science 156 (1985)] (Surface Science 156(1985)pp 678-700)), When golden particles are furthermore formed near the gold thin film, the surface plasmon of a thin film and the local surface plasmon of particles will do an interaction, and will have an absorption feature new as a complex of golden particles and a gold thin film. The reflection spectrum obtained by irradiating with the white light made parallel with a definite angle, and measuring catoptric light Ultraviolet, In the range of visible light and infrared wavelength, it has a remarkable absorption peak, and depends for the wavelength of said absorption peak in the kind of metal on a substrate, thickness and the particle diameter of polystyrene microspheres, the kind of metal vapor-deposited on adsorption density and polystyrene microspheres, and thickness notably. For example, if gold is vapor-deposited 20 nm in thickness to the polystyrene particles whose particle diameter is 55,110,152,209 nm, the sample which has a reflection spectrum shown in drawing 5 will be obtained.

[0011]That it is dependent on the dielectric constant in the 1/several less than field of particle diameter from an interface has a publicly known absorption spectrum of the isolated particles. Now, since golden particles are solid-phase-ized on the gold thin film in this patent and the local surface plasmon of golden particles and the surface plasmon of a gold thin film interact, compared with incident light, an electric field powerful several figures exists between particles and a thin film. When a dielectric constant changes all over an increase electric field, an absorption spectrum changes a lot. As an example, the spectrum in the air and an underwater spectrum are shown in drawing 6. Polystyrene microspheres with a particle diameter of 209 nm understand that an absorption-maximum value moves to 870 nm from the wavelength of 800 nm by replacing surface area with the water which has a different dielectric constant from air in the sample which vapor-deposited gold in thickness of 20 nm. Incidentally, change of a spectrum is quick and reversible and it is dependent on change of the dielectric constant in the field at several nanometers to several 100 nm from the surface.

[0012]Therefore, if biomolecules which divided the surface top of a substrate into two or more fields, and are different to each field, such as an antibody, an antigen, a single stranded DNA, receptor, and ligand, are made to adsorb, Since the reflectance in the specified wavelength which exists only in the field which combination produced changes, it becomes possible to detect adsorption, without using a fluorochrome.

[0013]One example of the biochemical sensor of example 1 this invention is shown in drawing 1. The substrate 1 is divided into two or more fields, and the polystyrene particles embellished with different biomolecule adsorb further in each field. For example, the polystyrene particles 3 which are sticking to the field 2 are embellished with the antibody 4, and the polystyrene particles 6 which are sticking to the field 5 are embellished with the antibody 7.

[0014]In order to detect analyte, the analyte which exists in a sample is beforehand embellished with the fluorochrome. If a sample is added to the substrate 1, the protein 8

which is the analyte which has specific binding ability to the antibody 4 will stick to the polystyrene particles 3, and the fluorochrome 9 will combine with the field 2. Or the antigen 10 which is the analyte which has specific binding ability to the antibody 7, When it combines with the polystyrene particles 6 by combination with said antibody 7, the fluorochrome 11 combines with the field 5 by adding the second antibody 12 which is embellished by the fluorochrome 11 and has a binding affinity to said antigen.

[0015]The substrate 1 is irradiated with the excitation light 14 from the light source 13 according to the optical system 15 after the end of joint of analyte. Since said fluorochrome 9 or said fluorochrome 11 is excited by this exposure and a fluorescence signal is emitted, this is condensed according to the optical system 16, and it monitors with the camera 17. Existence of the analyte in said sample can be distinguished by distinguishing the field which shows the wavelength of fluorescence, or a fluorescence.

[0016]An example of the method of the adsorption of particles to the substrate 1 is shown in drawing 7. A gold thin film prepares for the surface the substrate 1 currently formed by vacuum evaporation (a). Next, the template 40 by which two or more partitions were formed on this substrate 1 is placed (b). Subsequently, the polystyrene particles suspended in the carbodiimide liquid of 100mM from the concentration 1 are slushed into each partition (c). At this time, signs that suspension flows out of the crevice between the fields which touch the substrate 1 of the template 40 are shown in the right-hand side of drawing 7 (c). The substrate which adsorbed different polystyrene particles for every field further in this way can be formed (d).

[0017]Other examples of example 2 this invention are shown. Drawing 8 shows the principle of the method of patterning polystyrene particles by photo lithography. Drawing 8 (a) shows signs that the alkanethiol molecule 52 carries out self-organization to the surface of said gold thin film 51, and the monolayer 53 of an alkanethiol molecule is formed in it, by adding the solution of an alkanethiol molecule to the gold thin film 51 currently formed in the surface of the substrate 50. The monolayer 53 of an alkanethiol molecule becomes what the alkyl chain X54 with the terminal part Y55 arranged in parallel so that a part may be expanded and shown, but. The surface characteristic of a gold thin film is greatly influenced by the length of the alkyl chain X54 of the alkanethiol molecule 52 and the electric charge of the terminal part Y55, and relative-degree-of-intimacy aquosity.

[0018]It is known that the alkane thiol which carried out self-organization will exfoliate from a gold surface by the exposure of ultraviolet rays. Then, as shown in drawing 8 (b), by irradiating with the monolayer 53 of an alkanethiol molecule by the ultraviolet rays 56 selectively, the monolayer 53 is removed and the gold thin film 51 can be exposed selectively. Next, as shown in drawing 8 (c), the monolayer 58 of polystyrene particles is formed only in the field to which the gold thin film was exposed as shown in drawing 8 (d) by adding the polystyrene particles 57 suspended in the carbodiimide liquid of 50mM from the concentration 1 to a golden board.

[0019]Now, the patterning method of polystyrene particles based on the above-mentioned adsorption principle is shown in drawing 9. The gold thin film 60 prepares the substrate 59 currently formed in the surface. Subsequently, in order to prevent adsorption of the polystyrene particles to the surface of the gold thin film 60, said gold thin film 60 surface is embellished with the alkanethiol molecule 61 (a). Next, in order to adsorb polystyrene particles only in the specific region of the substrate 60, the surface of the substrate 59 embellished with the alkanethiol molecule in the surface via the optical system 64, the mask 65, and the optical system 66 is irradiated with the ultraviolet rays 63 irradiated from the light source 62. From the field 67 by which UV irradiation was carried out, since an alkanethiol molecule exfoliates, the surface of a gold thin film is exposed and the polystyrene particles 68 suspended in the carbodiimide solution stick to the pattern state of the mask 65 (b). The different polystyrene particles 71 in another field 70 are adsorbed by glaring using another mask 69 in the following stage (c). The substrate which repeated this procedure and adsorbed predetermined polystyrene particles at all the surfaces of the substrate 60 can be formed.

[0020]Other examples of example 3 this invention are shown. Drawing 10 explains other examples of this invention. On the flat silicon substrate 80, the gold thin film 81 5 to 1000 nm thick was first formed by vacuum evaporation. In order to make the surface of a gold thin film into hydrophilic nature, like sodium thioglycolate A carboxyl group, Or the gold thin film 81 was processed 1 minute or more with the alkanethiol solution (from concentration 0.01mM to 1M) which has an amino group like 2 **AMINO ethanethiol, and chemical modification was carried out in the alkanethiol molecule layer. Next, the layer of polystyrene particles was further formed in the gold thin film surface by adding on the surface the polystyrene microspheres 82 with a particle diameter of 5 nm to 100 micrometers suspended in the carbodiimide solution of the concentration 1-1M (a). Next, 100 nm of gold was vapor-deposited to the polystyrene particles made to adsorb from 5 nm in thickness, and the golden particles 83 were formed in them (b). The silicon substrate 80 by which the golden particles 83 were formed in the surface shows a remarkable development characteristic for the phenomenon originating in surface plasmon. If the refractive index in the surface of the golden particles 83 changes, an absorption spectrum will be considered as a shift. Since the aforementioned refractive index change is produced, arbitrary antigens are made to adsorb near the surface of the golden particles 83 by the following method.

[0021]In order to make the surface of the golden particles 83 into hydrophilic nature first, like sodium thioglycolate again A carboxyl group, Or the golden particles 83 were processed for 1 minute with the alkanethiol solution (from concentration 0.01mM to 1M) which has an amino group like 2 **AMINO ethanethiol, and chemical modification was carried out in the thiol molecule layer. Next, by suspending and adding the polystyrene particles 85 embellished with the antibody 84 to the carbodiimide solution of 1 to 50mM, the polystyrene particles 85 were adsorbed on the

surface of the golden particles 83 (c). The absorption spectrum of the golden particles 83 is shifted by this adsorption. Since an absorption spectrum will shift more if the antigen 86 furthermore combines with the antibody 84, the ligation reaction of the antibody 84 and the antigen 86 is detectable by the latter shift.

[0022]Also in this example, by using the patterning method of Example 1 or Example 2, the polystyrene particles embellished with a different antibody can be adsorbed for every field, and it irradiates with the silicon substrate 80 whole by white light on the silicon substrate 80 according to the method of drawing 1. By measuring the spectrum of the light which carries out regular reflection from each field, adsorption of an antigen and an antibody can be distinguished for every field.

[0023]Other examples of example 4 this invention are shown. The gold thin film 92 is formed on the polyethylene sheet 91 held at the frame 90. The following method is used in order to combine the polystyrene particles embellished with a DNA which is different to the field to which said polyethylene sheets 91 differ. As shown in drawing 11 (a), the ferromagnetic particles 93 are captured with the photo pincette 94, and it moves under the polyethylene sheet 91. Next, if the paramagnetism polystyrene particles 95 embellished with DNA are added from the polyethylene sheet 91, it will pull and gather for the ferromagnetic particles 93. The paramagnetism polystyrene particles 95 stick to the surface of the gold thin film 92 by adding the carbodiimide solution of 1 to 50mM (b). Next, the ferromagnetic particles 93 are moved to the field which changes with photo pincettes, and it gathers around said ferromagnetic polystyrene particle 94 by adding the paramagnetism polystyrene particles 96 embellished with another DNA. It can stick to a gold thin film [in / for said paramagnetism polystyrene particle 96 / another field of the polyethylene sheet 91] by adding the carbodiimide solution of 1 to 50mM again (c).

[0024]Since DNA which embellishes paramagnetism polystyrene particles for every magnetic particle is independently controllable by this example, it is advantageous to forming the ornamentation field of many analyte correspondences in a narrow field.

[0025]

[Effect of the Invention]By this invention, the simple sensor and sensing device which can detect much existence of analyte by high sensitivity simultaneously have been provided.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1]The figure showing how to detect the biochemical reaction in the array-ized particle surface.

[Drawing 2]The figure showing the detecting method in a DNA chip.
[Drawing 3]The figure showing the polystyrene particles combined on the hexagon-like gold pattern.
[Drawing 4]The figure showing the golden particles combined on the gold pattern of triangular shape.
[Drawing 5]The reflection spectrum of golden particles.
[Drawing 6]The figure showing the spectrum from which an absorption-maximum value changes with dielectric constants.
[Drawing 7]The figure showing how to patternize polystyrene particles using a template.
[Drawing 8]The figure showing the patterning principle by photo lithography.
[Drawing 9]The figure showing the procedure which patterns particles on a substrate.
[Drawing 10]The figure showing the particles combined near the golden particles.
[Drawing 11]The figure showing how to pattern the embellished particles by the ferromagnetic particles operated with the photo pincette.
[Description of Notations]
A substrate, 2:field, 3:polystyrene particles, 4:antibody, 5 : 1: A field, Polystyrene particles, 7:antibody, 8:protein, 9:fluorochrome, 10 : 6: An antigen, A fluorochrome, 12:second antibody, 13:light source, 14:excitation light, 15 : 11: An optical system, An optical system, 17:camera, 30:board, 31 : 16: A field, 32:DNA, A field, 34:DNA, 35:DNA fragment, 36 : 33: A confocal microscope, A fluorochrome, 38:fluorescence signal, 40:template, 50 : 37: A substrate, A gold thin film, 52:alkanethiol molecule, 53:monolayer, 54 : 51: An alkyl chain, A terminal part, 56:ultraviolet rays, 57:polystyrene particles, 58 : 55: A monolayer, A substrate, 60:gold thin film, 61:alkanethiol molecule, 62 : 59: The light source of ultraviolet rays, Ultraviolet rays, 64:optical system, 65:mask, 66:optical system, 67 : 63: A field, Polystyrene particles, 69:mask, 70:field, 71 : 68: Polystyrene particles, A silicon substrate, 81:gold thin film, 82:polystyrene particles, 83:gold particles, 84:antibody, 85:polystyrene particles, 86:antigen, 90:frame, 91:polyethylene sheet, 92:ferromagnetism particles, 93 : 80: A photo pincette, 94: Paramagnetism polystyrene particles, 95:ferromagnetism particles, 96 : paramagnetism polystyrene particles.